Amendments to the specification

Amend the paragraph starting at page 1, line 3, as follows.

This application is a continuation of U.S. Patent Application No. 09/294,457, filed April 19, 1999 (now U.S. Patent 6,348,567 ~~pending~~), which is a continuation-in-part of U.S. Patent Application No. 08/759,599, filed December 5, 1996 (now U.S. Patent 5,917,013), which claims benefit from U.S. Provisional Application 60/008,233, filed December 6, 1995 (now abandoned), each of which is hereby incorporated by reference.

A1

Amend the paragraph starting at page 2, line 9, as follows.

Apoptosis appears to be genetically regulated. However, apoptosis can be induced by exposing cells to radiation, heat, cytotoxic agents, and abnormal changes in cellular biology. The mitochondria may also be involved in apoptosis. Excessive cell death may result in crippling degenerative disorders, for example, the annihilation of vital $CD4^+$ T-lymphocytes in HIV (human immunodeficiency virus) infected patients; the elimination of neurons, and other cell types, following ischemia and reperfusion; and the destruction of cells after exposure to ionizing or ultraviolet radiation in the treatment of neoplastic disorders. These disorders are thought to stem from ectopically programmed cell death, e.g., metabolic or infective factors that induce the apoptosis. Too little cell death can result in proliferative disorders, such as neoplastic disorders or autoimmune disease when a particular immune cell lives beyond its appropriate life span.

A2

Amend the paragraph starting at page 4, line 3, as follows.

Neurodegenerative disorders include, by way of example, Parkinson's, Alzheimer's, Huntington's, cerebellar degeneration, and FALS (familial amyolateral sclerosis).

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Amend the paragraph starting at page 5, line 5, as follows.

A4

Figure 1 is a bar graph which depicts left ventricular developed pressure, i.e., the difference between peak systolic pressure and resting left ventricular pressure, in the isolated rat heart that was exposed to 45 minutes of ischemia by subjecting the heart to an 80% reduction in perfusion flow rate, under anoxic conditions (85% N_2 and 5% CO_2), followed by reperfusion at 15 ml/min. and reoxygenation. There is a more rapid recovery in the hearts that received the peptide (20 mer) (SEQ ID NO: 6) ~~(SEQ. ID. No. 6)~~ prior to reperfusion.

Amend the paragraph starting at page 5, line 12, as follows.

A5

Figure 2 is a bar graph which depicts survival of spinal cord cells exposed to ammonium persulfate, 1 mM for 2 hours (left) and for 1 hour (right). Cells pretreated with the 20 mer (SEQ ID NO: 6) ~~(SEQ. ID. No. 6)~~ had much better survival, i.e., less death. Indeed, the 20 mer almost completely prevented cell death, compared to the number of dead cells observed in the absence of ammonium persulfate.

Amend the paragraph starting at page 7, line 30, as follows.

A6

In a preferred embodiment of the present invention, suitable peptides may have the following core amino acid sequences: VAL-ASP-VAL (including--SER/TYR-VAL-ASP-VAL- (SEQ ID NO: 13) ~~(SEQ. ID. No. 13)~~; -VAL-ASP-VAL-GLU/ASP- (SEQ ID NO: 14) ~~(SEQ. ID. No. 14)~~; -SER/TYR-VAL-ASP-VAL-GLU/ASP- (SEQ ID NO: 15) ~~(SEQ. ID. No. 15)~~; and -VAL-ASP-VAL-GLU/ASP-TYR/THR- (SEQ ID NO: 16) ~~(SEQ. ID. No. 16)~~).

Amend the paragraph starting at page 8, line 4, as follows.

A7

Particularly preferred peptides in this regard include the following amino acid sequences:

a. SER-VAL-ASP-VAL-GLU-TYR ~~(SEQ. ID. No. 1)~~ (SEQ ID NO: 1)

b. TYR-VAL-ASP-VAL-ASP-THR ~~(SEQ. ID. No. 2)~~ (SEQ ID NO: 2)

c. THR-VAL-ASP-VAL-GLU-TYR ~~(SEQ. ID. No. 3)~~ (SEQ ID NO: 3)

d. TYR-VAL-ASP-VAL-ASP-THR-ASN-GLU-LEU-LEU-LYS ~~(SEQ. ID. No. 4)~~ (SEQ ID NO: 4)

e. SER-VAL-ASP-VAL-GLU-TYR-THR-VAL-GLN-PHE-THR-PRO-LEU-ASN-PRO-ASP-ASP-ASP ~~(SEQ. ID. No. 5)~~ (SEQ ID NO: 5)

A7 Cont'

f. SER-VAL-ASP-VAL-GLU-TYR-THR-GLN-PHE-THR-ASP-PHE-ARG-GLY-LYS-LEU-THR-LYS-LE U-LEU ~~(SEQ. ID. No. 6)~~ (SEQ ID NO: 6)

g. SER-VAL-ASP-VAL-GLU-TYR-THR-VAL-GLN-PHE-THR-PRO-LEU-ASN-PRO-ASP-ASP-ASP-PH E-ARG-PRO ~~(SEQ. ID. No. 7)~~ (SEQ ID NO: 7)

h. TYR-VAL-ASP-VAL-ASP-THR-ASN-GLU-LEU-LEU-LYS-SER-GLU-GLN-LEU-LEU-THR-ALA-SE R-GLU ~~(SEQ. ID. No. 8)~~ (SEQ ID NO: 8)

Amend the paragraph starting at page 8, line 20, as follows.

In the context of the present invention, the term "peptide" includes analogues and fragments thereof. The term "analogue" refers to any derivative of the peptide and peptides in which one or more amino acids have been Amendd with amino acids of similar size and charge, e.g., interchanging LEU and ILE or the attachment of another structure such as a cyclic compound or other molecule to the "peptide." Analogues also include peptides which contain one or more amino acids in an altered configuration (i.e., R or S; or, L or D). The term "fragment" refers to any fragment of the peptide which is capable of ameliorating cell death as described above. Preferably, fragments are at least four amino acids in length; even more preferably, fragments are at least six amino acids in length ~~(see eg., SEQ I.D. Nos. 13, 14, 15 and 16)~~ (see *e.g.*, SEQ ID NOs: 13, 14, 15 and 16).

A8

Amend the paragraph starting at page 12, line 4, as follows.

Another aspect of the present invention provides polynucleotides which encode the above described peptides, analogues or fragments thereof. Polynucleotides and analogues thereof include, by way of example, RNA, DNA analogues thereof, including chimerics and PNA (peptide nucleic acids). The polynucleotides of the present invention may be synthesized or isolated. Synthesis may be accomplished using any one of several means including standard polynucleotide synthesis procedures. The polynucleotides coding for the aforementioned peptides could either be inserted into a standard plasmid or viral vector, introduced into bacterial or eukaryotic cells and the peptides of the present invention expressed and isolated.

A 9

Amend the paragraph starting at page 13, line 16, as follows.

Promoter regions may be selected from any desired gene using chloramphenicol transferase ("CAT") vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_{RO} and trc. Eukaryotic promoters include CMV (cytomegalovirus) immediate early, HSV (herpes simplex virus) thymidine kinase, early and late SV40, LTRs from retrovirus and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of one of ordinary skill in the art.

A 10

Amend the paragraph starting at page 13, line 23, as follows.

In a further embodiment, the present invention provides host cells containing the above-described construct. The host cell can be a eukaryotic cell, for example, a mammalian cell or a yeast cell; or a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be affected using any one of several methods known in the art, including by calcium phosphate transfection, DEAE (diethylaminoethyl), dextran mediated transfection, infection,

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A 11 Cont

or electroporation, as described in detail in, e.g., Davis et al., Basic Methods of Molecular Biology, 1986.

Amend the paragraph starting at page 19, line 12, as follows.

Compounds of the present invention may also be administered to treat a warm-blooded animal which has been subjected to a procedure with which cell death is associated. Such procedures include, by way of example, cardiac catherization, bypass surgery, chemotherapy, and chemically-induced reperfusion. This association is determined by clinical examination and appropriate testing, depending on the organ. By way of example, such tests include, echocardiograms, electrocardiograms, nuclear studies, and biochemical tests, e.g., CK (creatine kinase), and CK-MB (creatine kinase-MB).

A 12

Amend the paragraph starting at page 19, line 19, as follows.

Compounds of the present invention may also be administered to treat a warm-blooded animal which has been administered therapeutics which subject the animal to oxidative stress. The free radical production associated with oxidative stress may be identified and evaluated to ascertain the effect of the therapeutics using any suitable method, including thiobarbaturic acid, colormetric assays (TBARS), and spin resonance. Such therapeutics include, by way of example, clozapine, AZT (azidothymidine), and anthracyclines.

A 13

Amend the paragraph starting at page 23, line 4, as follows.

The HPLC-purified peptides eluted in fractions 12 and 13 were analyzed by amino acid analysis (R. L. Heinriksen and S. C. Meredith, Anal. Biochem. 160:65-74, 1984) after gas phase sequencing (N. M. Meltzer et al., Anal. Biochem. 160:356-61, 1987). The sequence of the purified peptide was determined by Edman degradation on a commerically available sequencer (R. M. Hewick et al., J. Biol. Chem. 15:7990-8005, 1981). The sequences were:

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fraction 12: YVDVDTNELLKSEQLLTASE ~~(SEQ. ID NO. 8)~~ (SEQ ID NO: 8)

A14 cont

fraction 13: SVDVEYTVQFTPLNPDDDFRP ~~(SEQ. ID NO. 7)~~ (SEQ ID NO: 7)

Amend the paragraph starting at page 24, line 6, as follows.

Based on the sequences identified in Example 2, peptides were synthesized using a commercially available automated synthesizer (Applied Biosystems 430 A Peptide Synthesizer), purified by and tested according to the assay described in Example 1 for their ability to ameliorate cell death in cardiac myocytes. The sequences for these peptides were:

6 mer #1: SVDVEY ~~(SEQ. ID NO. 1)~~ (SEQ ID NO: 1)

6 mer #2: YVDVDT ~~(SEQ. ID NO. 2)~~ (SEQ ID NO: 2)

6 mer #3: TVDVEY ~~(SEQ. ID NO. 3)~~ (SEQ ID NO: 3)

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11 mer: YVDVDTNELLK ~~(SEQ. ID NO. 4)~~ (SEQ ID NO: 4)

18 mer: SVDVEYTVQFTPLNPDDD ~~(SEQ. ID NO. 5)~~ (SEQ ID NO: 5)

20 mer: SVDVEYTQFTDFRGKLTKLL ~~(SEQ. ID NO. 6)~~ (SEQ. ID NO. 6)

fraction 12: YVDVDTNELLKSEQLLTASE ~~(SEQ. ID NO. 8)~~ (SEQ ID NO: 8)

fraction 13: SVDVEYTVQFTPLNPDDDFRP ~~(SEQ. ID NO. 7)~~ (SEQ ID NO: 7)

Irrelevant #1: NFLRGKLKLYTGEACRTGDR ~~(SEQ. ID NO. 9)~~ (SEQ ID NO: 9)

Irrelevant#2: RLILDSRVLERYLLEAKEAE ~~(SEQ.ID. NO. 10)~~ (SEQ ID NO: 10)

Irelevant #3: EVTEEEETVPLKThE-AMIDE ~~(SEQ. ID NO. 11)~~ (SEQ ID NO: 11)

Amend the paragraph starting at page 26, line 21, as follows.

One group of isolated rat hearts was pretreated with a 20 mer ~~(SEQ. ID NO. 6)~~ (SEQ ID NO: 6). The 20 mer ~~(SEQ. ID NO. 6)~~ (SEQ ID NO: 6) was added to the perfusate and hearts were perfused starting 15 minutes before reperfusion and continuing for 5-10 minutes after reperfusion. Left ventricular developed pressure was measured and compared to a control group of isolated rat hearts receiving no pretreatment. Left ventricular developed pressure, an index of left ventricular performance, is the difference between peak systolic pressure and resting left ventricular pressure. The results of this experiment are plotted in Figure 1. Hearts pretreated with the 20 mer peptide ~~(SEQ. ID NO. 6)~~ (SEQ ID NO: 6) experienced a rapid recovery.

A16

Amend the paragraph starting at page 27, line 18, as follows.

Both the experimental and control cultures were treated with 1 mM ammonium persulfate for 1 or 2 hours at 37°C. The experimental cultures were treated with either 10 μg/ml or 20 μg/ml of the 20 mer peptide ~~(SEQ. ID NO. 6)~~ (SEQ ID NO: 6) for the same length of time. Results of two experiments, each carried out in duplicate, are shown in FIG. 2. The number of dead cells, assayed by trypan blue, in the experimental groups was compared to that in the control group. Pretreatment with the 20 mer peptide ~~(SEQ. ID NO. 6)~~ (SEQ ID NO: 6) dramatically enhanced cell survival.

A17

Amend the paragraph starting at page 28, line 7, as follows.

Cells in the latter group were washed free of the 20 mer (SEQ. ID NO. 6), given saturating concentrations of growth factor (5 ng/ml of human interleukin-3) and incubated for an additional 22 hours. After the 22 hours, 1 μCi of ^{3}H-thymidine (2 Ci/mmol) was added and, after 2 hours, the cellular contents were harvested onto filtermats. Then ^{3}H-thymidine incorporation was measured using an LKB Betaplate Harvester and liquid scintillation counter. The results of this

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A18 Cont

experiment are presented in Tables 4 and 5. Note the increased viability of cells treated with the 20 mer peptide ~~(SEQ. ID NO. 6)~~ (SEQ ID NO: 6).